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RNA processing

1. Introduction
2. Mechanisms of RNA processing
3. Processing of pre-mRNA
4. 5’ capping
5. Polyadenylation
6. Types of polyadenylation
7. Intron splicing
8. Exon and intron Definition
9. Splicing apparatus
10. Alternative splicing
11. Trans splicing
12. RNA editing
13. Processing of pre-rRNA
14. Group I and group II introns
15. Processing of pre-tRNA
16. mRNA degradation
17. mRNA surveillance

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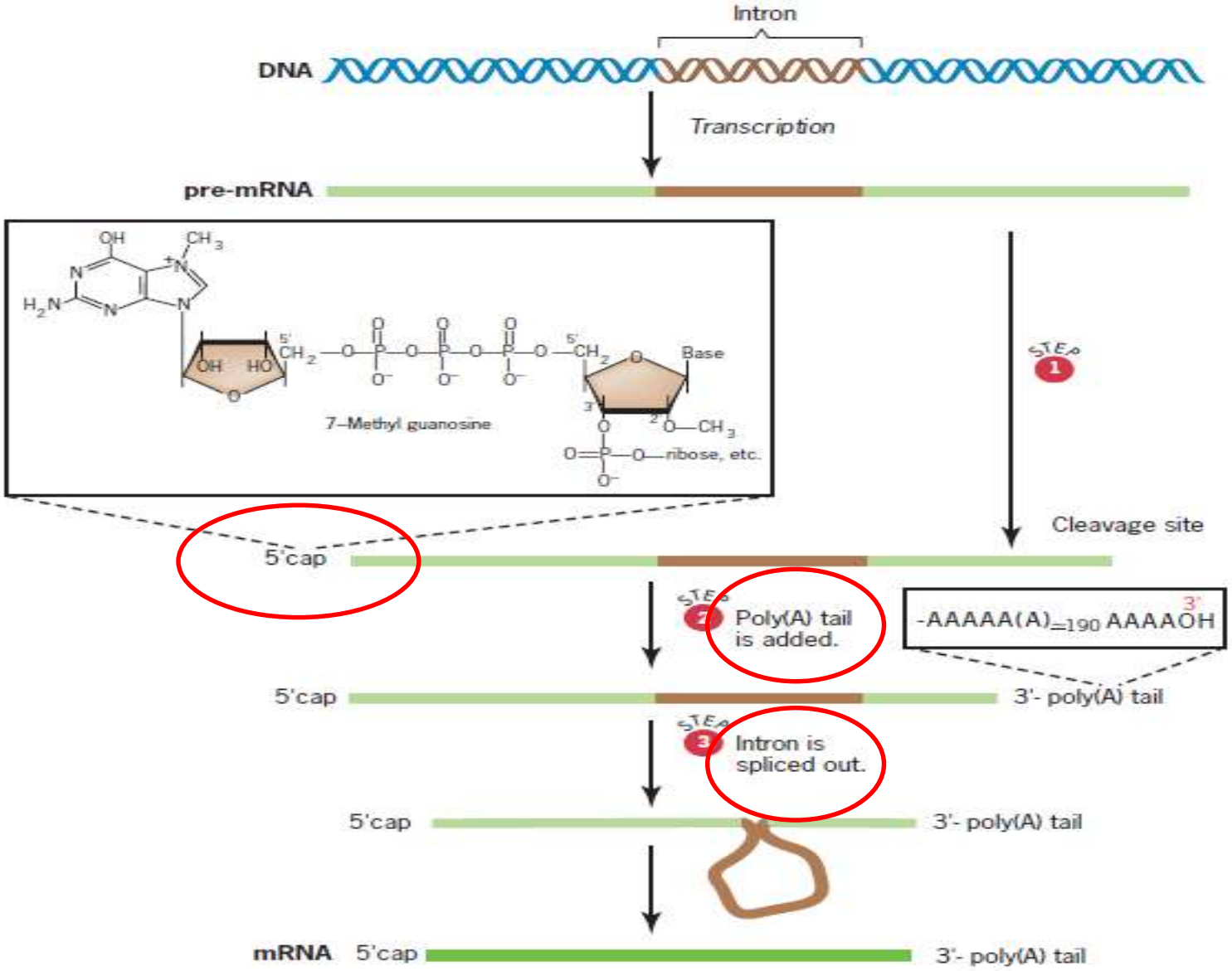

■ **FIGURE 11.12** In eukaryotes, most gene transcripts undergo three different types of posttranscriptional processing.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 268

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In **eukaryotes**,

- Transcription and translation takes place in different cellular components
- Transcription takes place in nucleus; translation happens in cytoplasm
- Thus mRNA undergoes processing/ modifications like- cleavage, addition of nucleotides and chemical modification after synthesis

In **prokaryotes**,

- Transcription of mRNA and translation occur simultaneously
- Thus mRNA undergo little or no modification after synthesis by RNA polymerase

Both prokaryotes and eukaryotes modify pre-tRNA, Pre-rRNA
Eukaryotes very extensively process pre-mRNA destined to become mRNA

Primary transcript of RNA polymerase = pre-RNA → Processing if pre-RNA (especially mRNA)

- 5’ capping
- 3’ cleavage/ polyadenylation
- Splicing
- RNA editing

Pre-RNA —Processing→ Processed RNA → Transported to cytoplasm → Translated by ribosomes

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Processing of eukaryotic pre-mRNA

1. 5’ Capping
2. Polyadenylation
3. Splicing
4. RNA editing

Processing of eukaryotic pre-rRNA

1. Cutting and end trimming process
2. Chemical modification of rRNA
3. Splicing

Processing of pre- tRNA

1. Cleavage
2. Trimming
3. Chemical modification
4. Splicing

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Processing of eukaryotic pre-mRNA

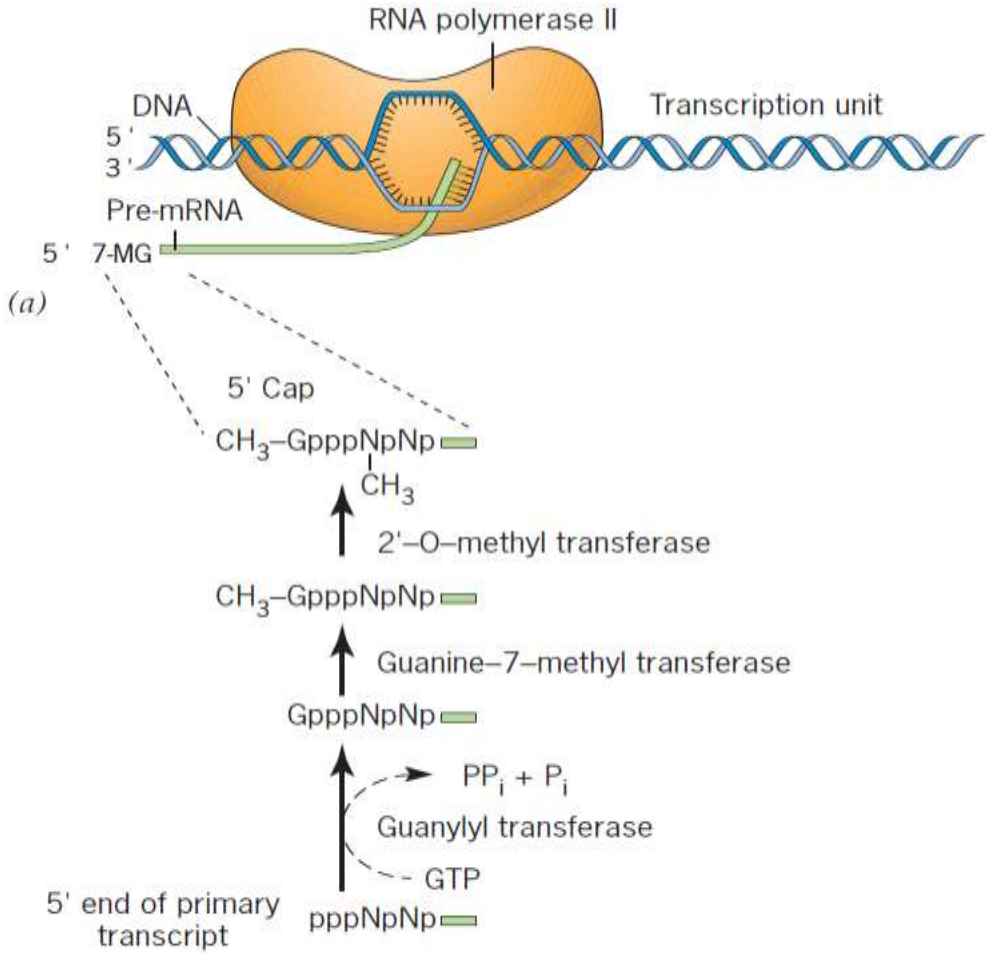
1. 5’ Capping:

- Eukaryotic mRNA consists of enzymatically appended cap structure consisting of 7-methylguanosine residue joined via 5’-5’ triphosphate bridge
- Capping of pre-mRNA is catalyzed by enzyme RNA polymerase II

Functions of 5’ capping:

- Protection of mRNA from degradation
- Transport of the mRNA from nucleus to cytoplasm
- Binding of ribosome with mRNA

Early stage in the transcription of a gene by RNA polymerase II.



(b) **Pathway of biosynthesis of the 7-MG cap.**

■ **FIGURE 11.16** 7-Methyl guanosine (7-MG) caps are added to the 5′ ends of pre-mRNAs shortly after the elongation process begins.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 272

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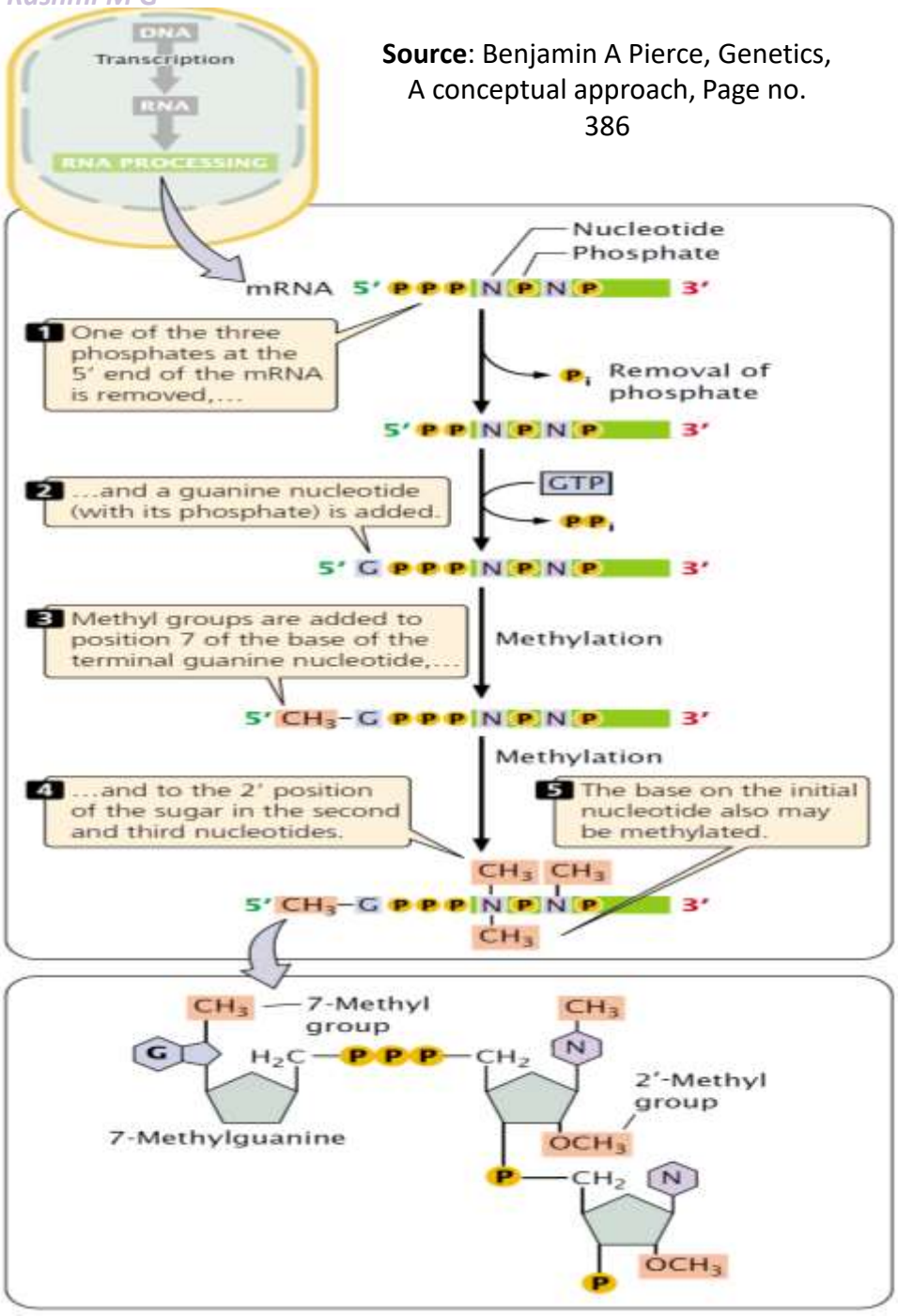
DNA
Transcription
RNA
RNA PROCESSING
Nucleotide
Phosphate
mRNA 5′ P P P N P N P 3′
1 One of the three phosphates at the 5′ end of the mRNA is removed,...
Pi Removal of phosphate
5′ P P N P N P 3′
2 ...and a guanine nucleotide (with its phosphate) is added.
GTP
PPi
5′ G P P P N P N P 3′
3 Methyl groups are added to position 7 of the base of the terminal guanine nucleotide,...
Methylation
5′ CH3–G P P P N P N P 3′
Methylation
4 ...and to the 2′ position of the sugar in the second and third nucleotides.
5 The base on the initial nucleotide also may be methylated.
CH3 CH3
5′ CH3–G P P P N P N P 3′
CH3
CH3 7-Methyl group
G
H2C P P P CH2
N
CH3
2′-Methyl group
OCH3
7-Methylguanine
P CH2 N
OCH3
P

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 386

During transcription, 7-methylguanosine is added to the 5’ end of nascent mRNA

↓

One subunit of Capping enzyme removes the γ-Phosphate from the 5’ end of the nascent RNA

↓

The other subunit transfers the GMP moiety from GTP to the 5’-Diphosphate of the nascent transcript creating the guanosine 5’-5’ triphosphate structure

↓

Separate enzymes transfer methyl groups from S-adenosylmethionine to the N^7 position of the guanine at the 5’ end of the nascent RNA

↓

If mRNA has a methyl group on N7 position of the guanine at the 5’ end, then it is called cap 0

↓

This is the first methylation step and occurs in all eukaryotes

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2. Polyadenylation:

- Eukaryotic mRNAs have a series of upto 250 adenosines at their 3' end called poly (A) tail
- A poly (A) tail is added to the 3' end of the mRNA and plays an important role in mRNA stability, nucleocytoplasmic export and translation
- Poly (A) tail is not specified by the DNA and is added to the transcript by a template – independent RNA polymerase called **poly (A) polymerase** after endonucleolytic cleavage of the nascent RNA near the 3' terminus
- A single processing complex undertakes both cleavage and polyadenylation
- A signal is needed for both cleavage and polyadenylation

In mammals, signal sequence called **poly (A) signal** in the mRNA located between 10-30 nucleotides upstream of the polyadenylation site

<30 nucleotides downstream of the polyadenylation site is **GU rich downstream element**

Both are binding sites for multi-subunit protein complexes :

Cleavage and polyadenylation specificity factor (CPSF)
Cleavage stimulation factor (CstF)

Generation of poly (A) tail at 3' terminus first requires cleavage factors: **CFI** and **CFII** –acts as endonucleases to cleave the RNA at polyadenylation site- Cleavage step

Poly (A) polymerase (PAP) catalyzes the polyadenylation reaction in template independent manner
Its substrate is ATP

Polyadenylation reaction passes through 2 stages:

- Rather short oligo (A) sequence is added to the 3' end
- Oligo (A) tail is extended to the full residue length
- This reaction requires another stimulatory factor that recognizes the oligo (A) tail= **Poly Adenylate Binding Protein (PABP)** which helps the polymerase to add the adenosines, possibly influences the length of the poly (A) tail that synthesized and appears to play a role in the maintenance of the tail after synthesis

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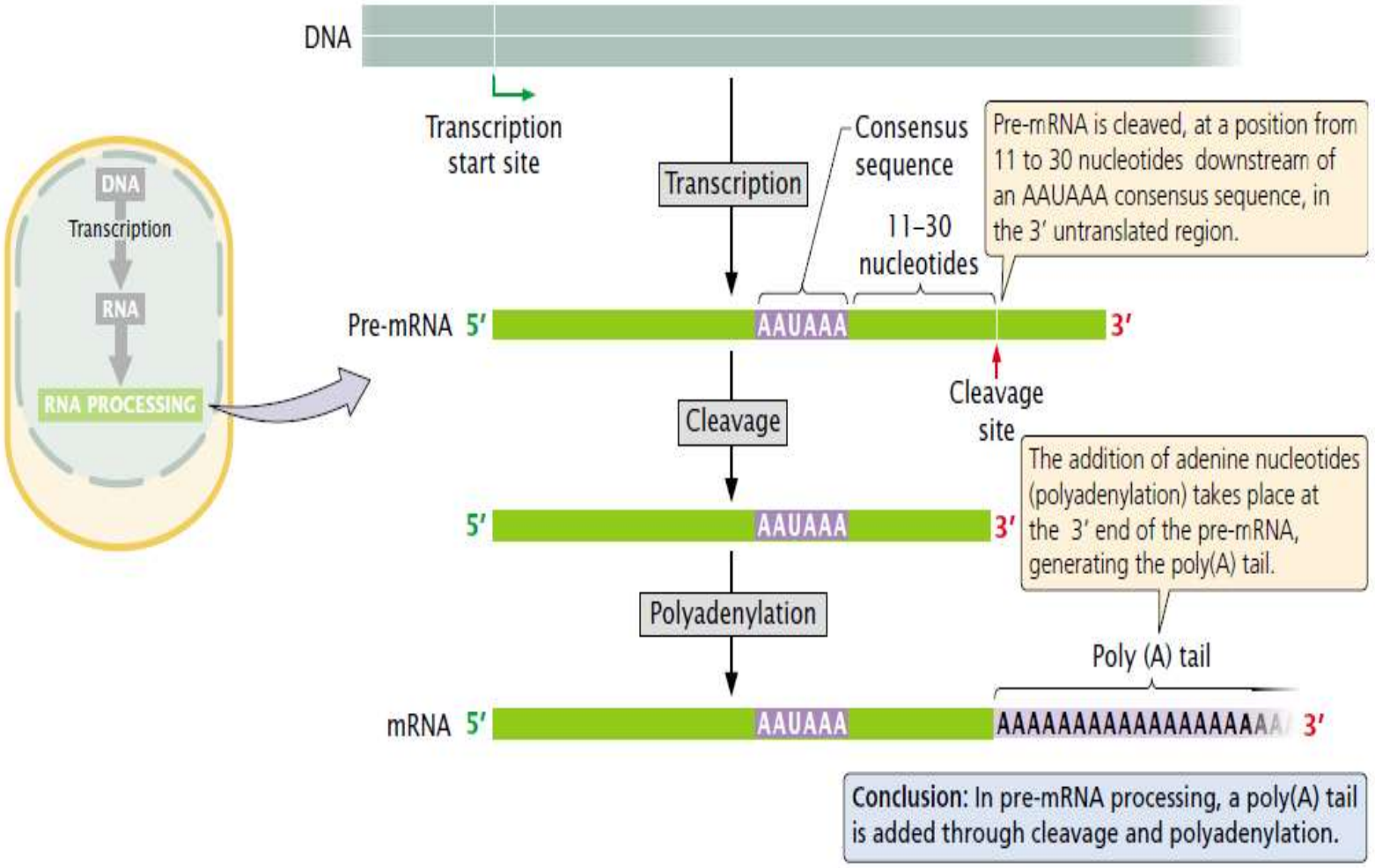
DNA
Transcription start site
Transcription
Consensus sequence
11–30 nucleotides
Pre-mRNA is cleaved, at a position from 11 to 30 nucleotides downstream of an AAUAAA consensus sequence, in the 3′ untranslated region.
DNA
Transcription
RNA
RNA PROCESSING
Pre-mRNA 5′ AAUAAA 3′
Cleavage site
Cleavage
5′ AAUAAA 3′
The addition of adenine nucleotides (polyadenylation) takes place at the 3′ end of the pre-mRNA, generating the poly(A) tail.
Polyadenylation
Poly (A) tail
mRNA 5′ AAUAAA AAAAAAAAAAAAAAAAAA 3′
Conclusion: In pre-mRNA processing, a poly(A) tail is added through cleavage and polyadenylation.

14.7 Most eukaryotic mRNAs have a 3′ poly(A) tail.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 387

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Types of polyadenylation

Alternative polyadenylation

- Occurs in mRNA of some protein coding genes
- Addition of poly (A) tail at several possible sites
- These type of genes have more than one polyadenylation site
- Causes the formation of more than one transcript from a single gene
- Ex. Alternative polyadenylation occurring during the development of B-lymphocytes

Cytoplasmic polyadenylation

- Targets mRNA that already contains a short poly (A) tail
- Increases the length of the short poly (A) tail before translation
- Length of poly (A) tail can be regulated in the cytoplasm
- Stored mRNA has a short poly (A) tail
- Activation of the mRNA for translation includes lengthening of the poly (A) tail
- Catalyzed by a **cytoplasmic poly (A) polymerase**
- Ex. Egg cell stored mRNA in the cytoplasm for later use after the fertilization

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3. Intron splicing:

- Noncoding DNA is also found within most eukaryotic genes
- Such genes have a split structure in which segments of coding sequence (called expressing sequences or **exons**) are separated by noncoding sequences (intervening sequences or **introns)**
- The entire gene is transcribed to yield a long RNA molecule and the introns are then removed by splicing, so only exons are included in the mRNA
- The process of excising the sequences in RNA that corresponds to introns and joining of sequences corresponding to exons is called **RNA splicing**

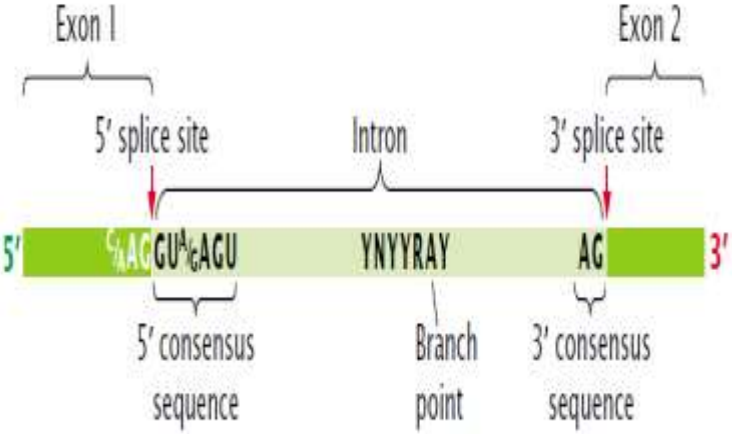
Types of pre-mRNA introns splicing

GU-AG introns splicing

In GU-AG intron, the 1st two nucleotides of the intron sequence are 5'-GU-3' (5' splice site or donor site) and the last two 5'-AG-3' (3' splice site or acceptor site

AU-AC introns splicing

AU-AC introns are rare class of introns, because the conserved 5' GU and 3' AG dinucleotides are replaced by AU and AC respectively



14.10 Splicing of pre-mRNA requires consensus sequences. In the consensus sequence surrounding the branch point (YNYYRAY) Y is any pyrimidine, R is any purine, A is adenine, and N is any base.

Conclusion: Critical consensus sequences are present at the 5′ splice site, the branch point, and the 3′ splice site.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 389

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Transesterification reactions:
Splicing of GU-AG introns involves 2 transesterification reactions

Cleavage of the 5’ splice site occurs by the first transesterification reaction promoted by the hydroxyl group attached to the 2’ carbon of an adenosine nucleotide located within the intron sequence

↓

The result of the hydroxyl attack is cleavage of the phosphodiester bond at the 5’ splice site, accompanied by formation of a new 5’-2’ phosphodiester bond linking the first nucleotide of the intron (the G of the 5’-GU-3’ motif) with the internal adenosine

↓

This means that the intron has now been looped back on itself to create **lariat structure**

↓

Cleavage of the 3’ splice site and joining of the exons result from a second transesterification reaction, promoted by the 3’-OH group attached to the end of the upstream exon

↓

The group attacks the phosphodiester bond at the 3’ splice site, cleaving it and so releasing the intron as the lariat structure, which is subsequently converted back to a linear RNA and degraded

↓

At the same time, the 3’ end of the upstream exon joins the newly formed 5’ end of the downstream exon, completing the splicing process

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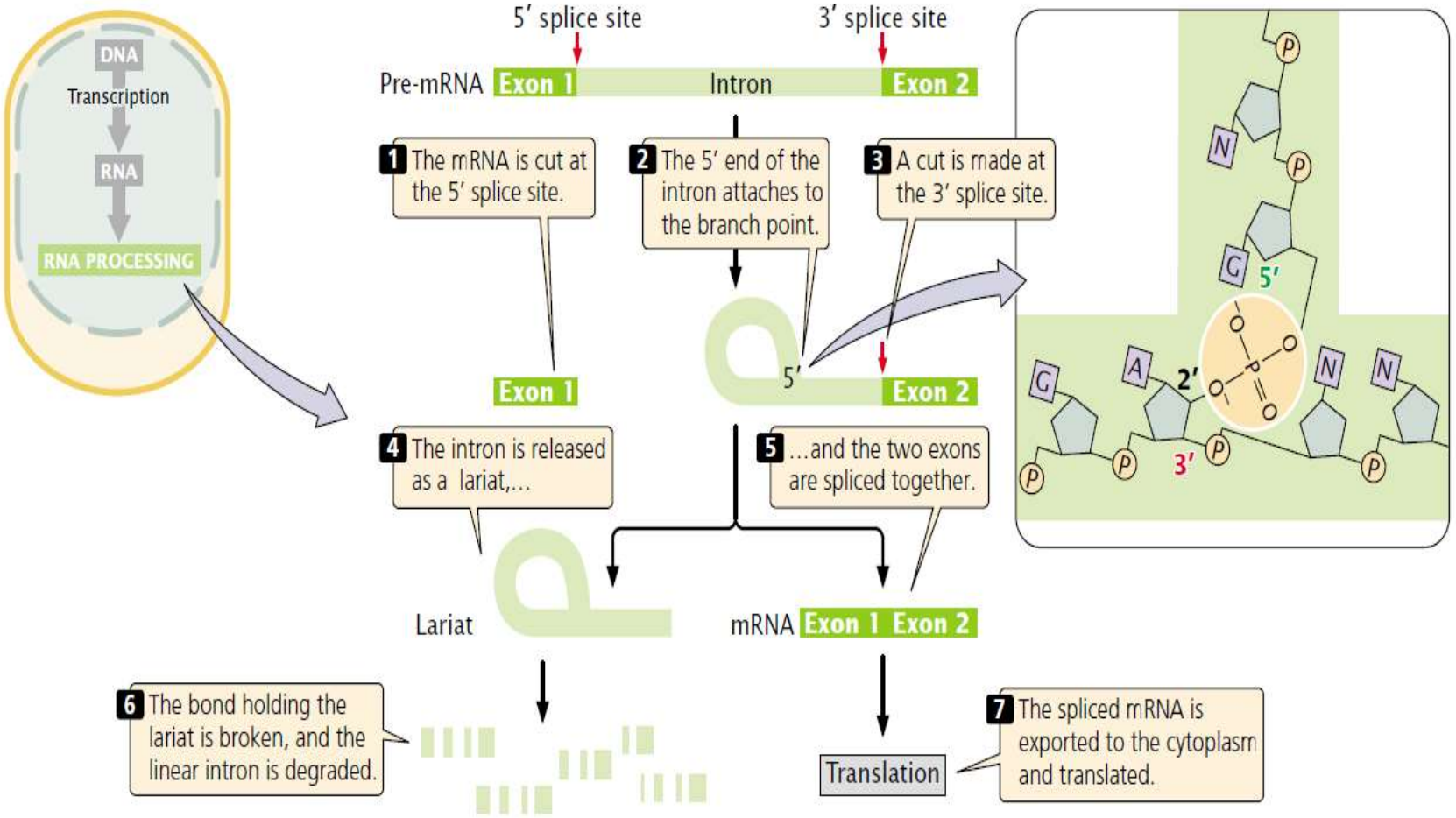

14.11 The splicing of nuclear introns requires a two-step process. First, cleavage takes place at the 5′ splice site, and a lariat is formed by the attachment of the 5′ end of the intron to the branch point. Second, cleavage takes place at the 3′ splice site, and two exons are spliced together.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 389

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Exon definition and Intron Definition:

In **exon definition**: splicing factors recognize the ends of the exons and splice out the introns in between

This mechanism is not universal and occurs when the introns are long and the splice sites are weak

In **intron definition**: the ends of introns are recognized

In this case, two splice sites are recognized without requiring any sequences outside the intron

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Splicing apparatus:

- The central components of the splicing apparatus for GU-AG introns are the **snRNAs** called U1,U2,U4,U5 and U6
- These short RNA molecules associate with proteins to form **small nuclear ribonucleoproteins (snRNPs)**
- Cajal bodies within the nucleus are centers of post-transcriptional modifications of snRNAs and snRNPs assembly
- snRNPs+ other accessory proteins+ attached to transcript= forms a series of complex, the last complex= **Spliceosome**
- The structure within which the actual splicing reaction occur

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Process of assembly of snRNPs and various protein factors:

Commitment complex (E complex) initiates a splicing activity

This complex comprises of:

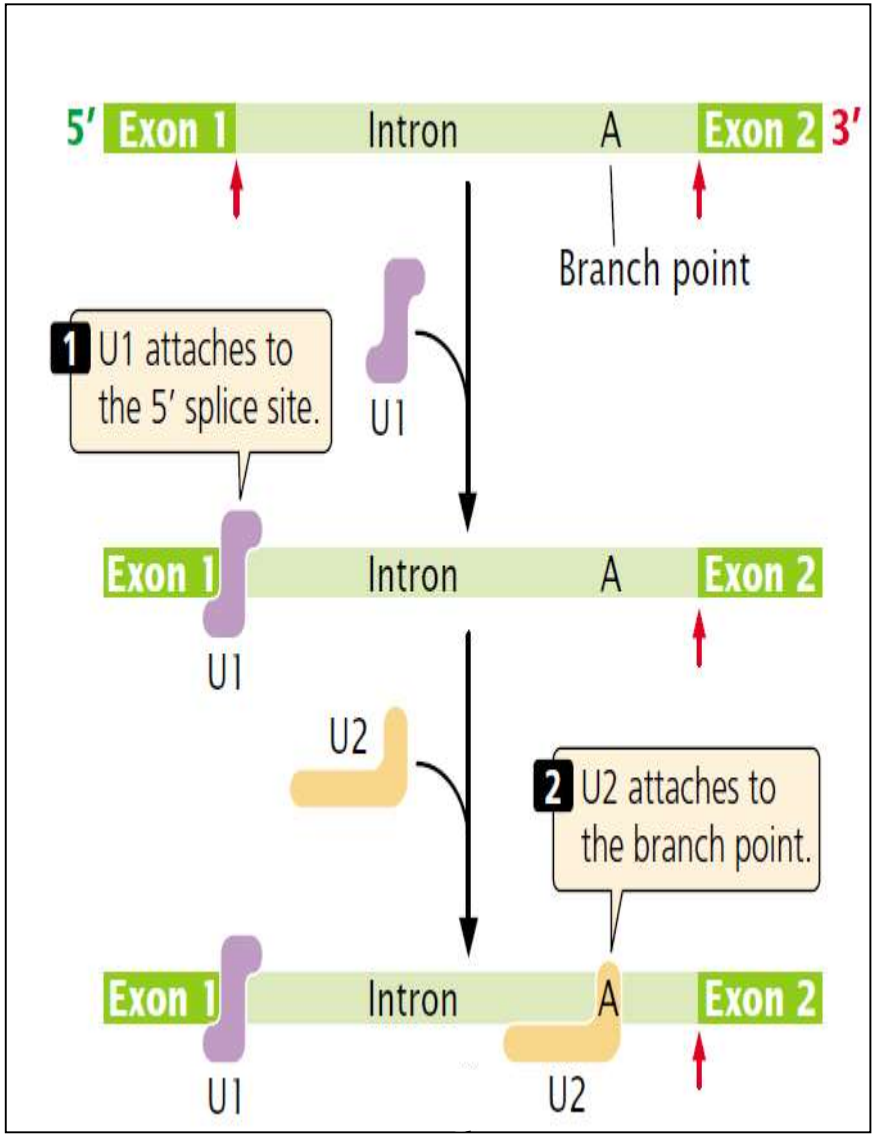
U1-snRNP, which binds to the 5' splice site by RNA-RNA base pairing (1st step in splicing)

Branch point Binding Protein (BBP), which binds branch point, the splicing factor **U2AF**, binds with the polypyrimidine tract and members of SR protein family

SR protein interact with one another via their Arg-Ser-rich regions and also bind to RNA

↓

U2-snRNP binds to branch site (**E complex** is converted to the **A complex**)



Pre-Spliceosome complex (**A complex**) comprises of: **Commitment complex** + **U2snRNP**, the latter attached to the branch site

At this stage, an association between **U1-snRNP** and the **U2-snRNP** brings the 5' splice site into close proximity to the branch point

↓

The **Spliceosome** is formed when **U4/U6-snRNP** (a single snRNP containing two snRNAs) and **U5-snRNP** attach to the **pre-Spliceosome complex**

↓

The other **snRNPs** involved in splicing associate with the complex in a defined order

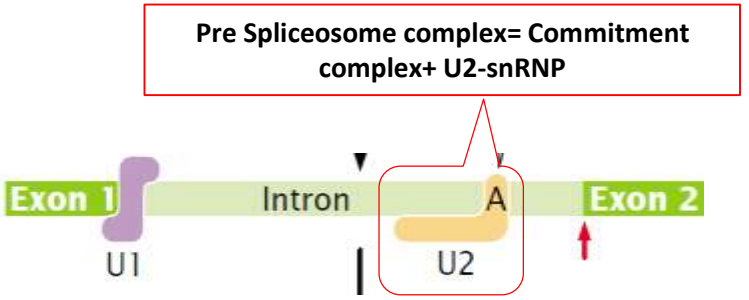


Table 14.2 RNA–RNA interactions in pre-mRNA splicing

Interaction	Function
U1 with 5′ splice site	U1 attaches to 5′ end of intron; commits intron to splicing; no direct role in splicing
U2 with branch point	Positions 5′ end of intron near branch point for lariat formation
U2 with U6	Holds 5′ end of intron near branch point
U6 with 5′ splice site	Positions 5′ end of intron near branch point
U5 with 3′ end of first exon	Anchors first exon to spliceosome subsequent to cleavage; juxtaposes two ends of exon for splicing
U5 with 3′ end of one exon and 5′ end of the other	Juxtaposes two ends of exon for splicing
U4 with U6	Delivers U6 to intron; no direct role in splicing

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B1 complex formed when,
U5 and **U4/U6- snRNPs** binds to complex
This complex is regarded as a **Spliceosome**, since it contains the components needed for the splicing reaction

↓

U1-snRNP released
B1 complex is converted into **B2 complex**

↓

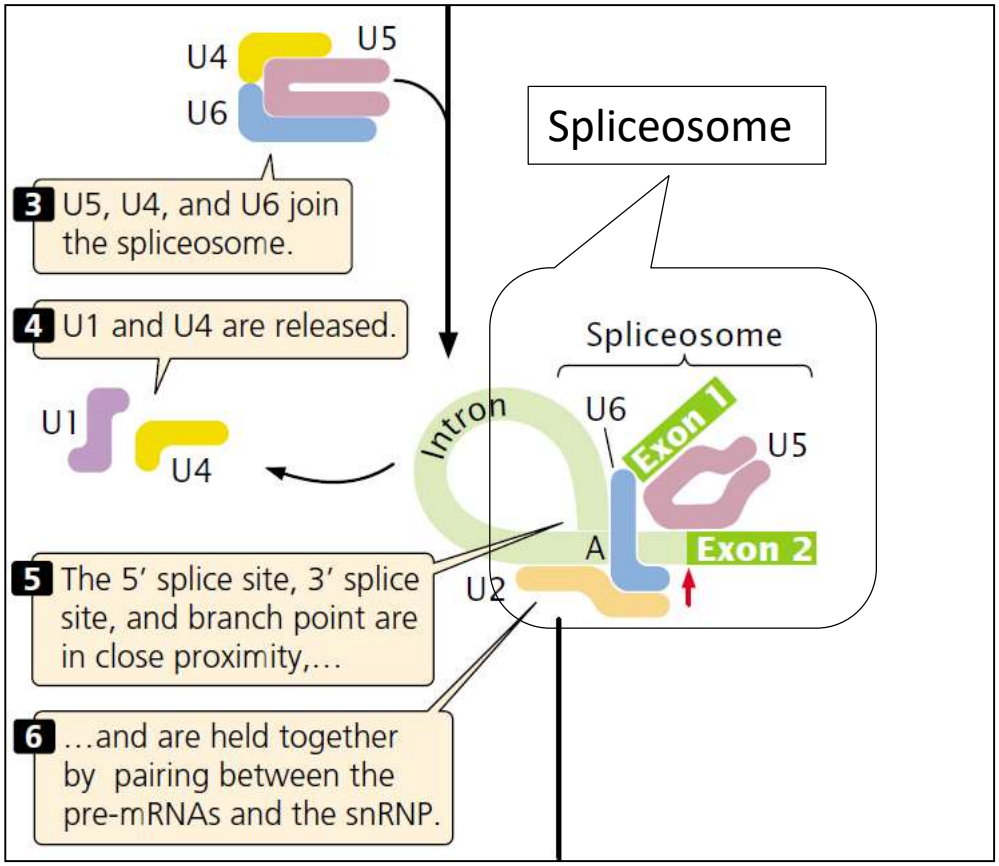
The release of **U1-snRNP** allows **U6-snRNP** to interact with the 5' splice site

↓

The catalytic reaction is triggered by the release of **U4**
This requires hydrolysis of ATP

↓

When **U4** dissociates from **U6-snRNP**, **U6-snRNA** can pair with **U2-snRNA** to form the catalytic active site (**C complex**)
This results in additional interactions that bring the 3' splice site close to the 5' site and the branch point



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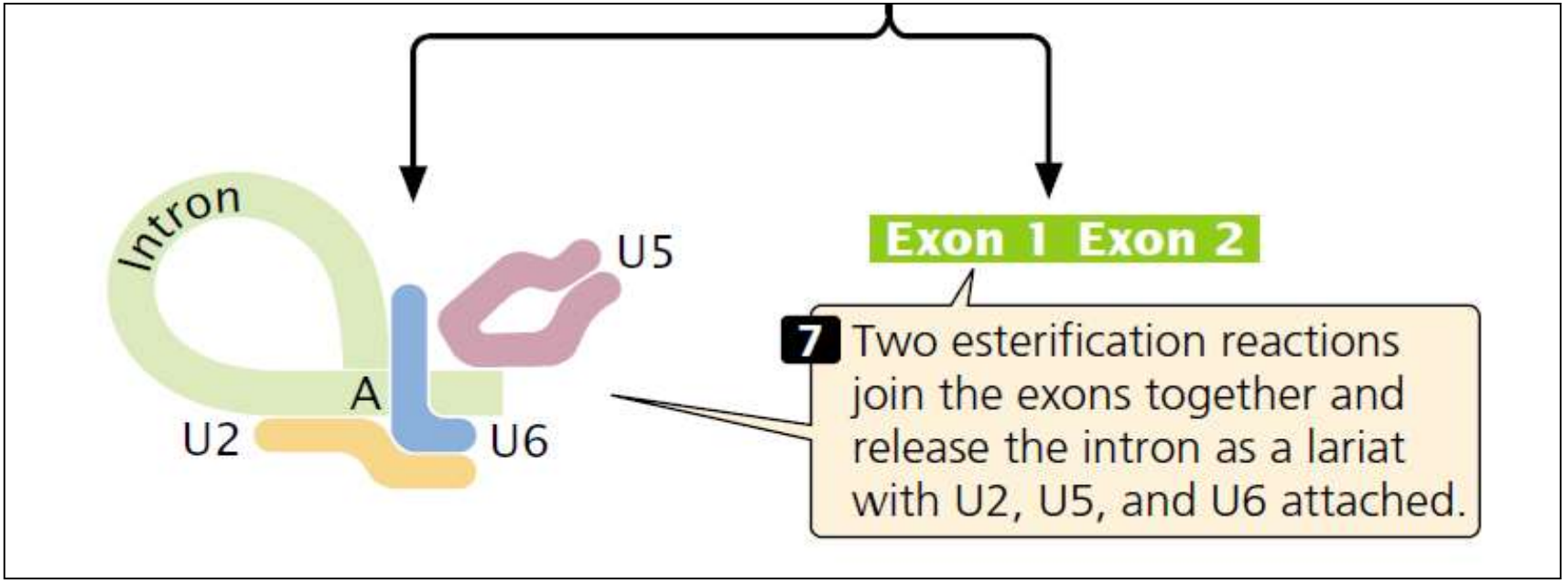
All three key positions in the intron are now in proximity and the 2nd transesterification occur as a linked reaction, possibly catalyzed by **U6-snRNP**, completing the splicing process

↓

The two exon sequences are, thereby joined to each other and the intron sequence is released as a lariat

↓

ATP is required for assembly of the Spliceosome, but the transesterification reactions do not require ATP


Intron
U5
A
U2
U6
Exon 1 Exon 2
7 Two esterification reactions join the exons together and release the intron as a lariat with U2, U5, and U6 attached.

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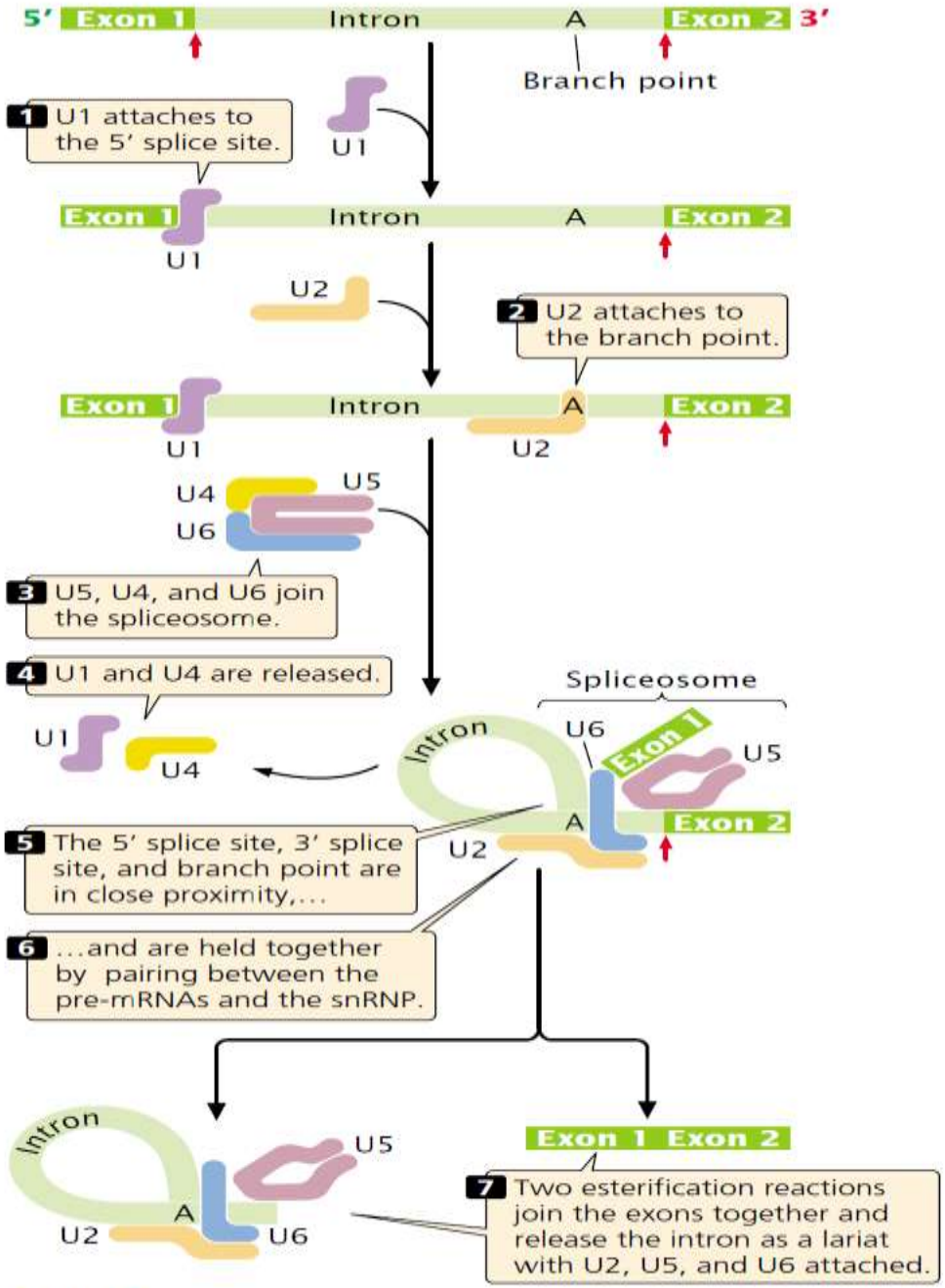

14.12 RNA splicing takes place within the spliceosome.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 360

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Alternative splicing

- In some cases alternative 5’ and/or 3’ splice sites are used during splicing
- This results in the production of more than one mRNA species from a single pre-mRNA
- The production of different RNA products from a single product by changes in the usage of splicing junctions is called Alternative splicing
- Ex. ***Drosophila*** sex determination

Trans splicing

- In this case exons from two separate RNA transcripts are spliced together to form a mature mRNA molecule
- All the products of trans-splicing have the same 5’ exon and different 3’ exons
- Ex. Single celled ***trypanosomes***

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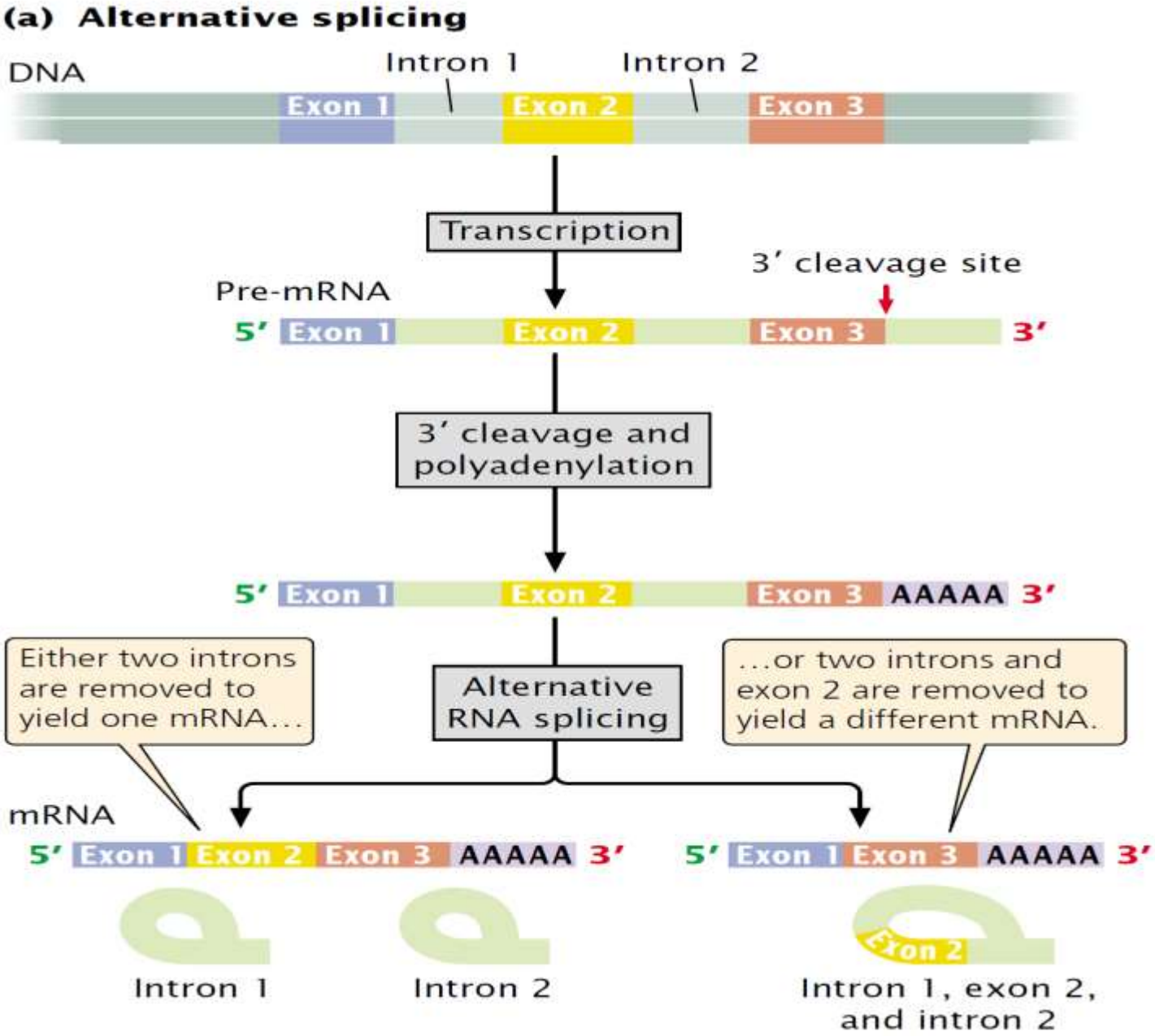
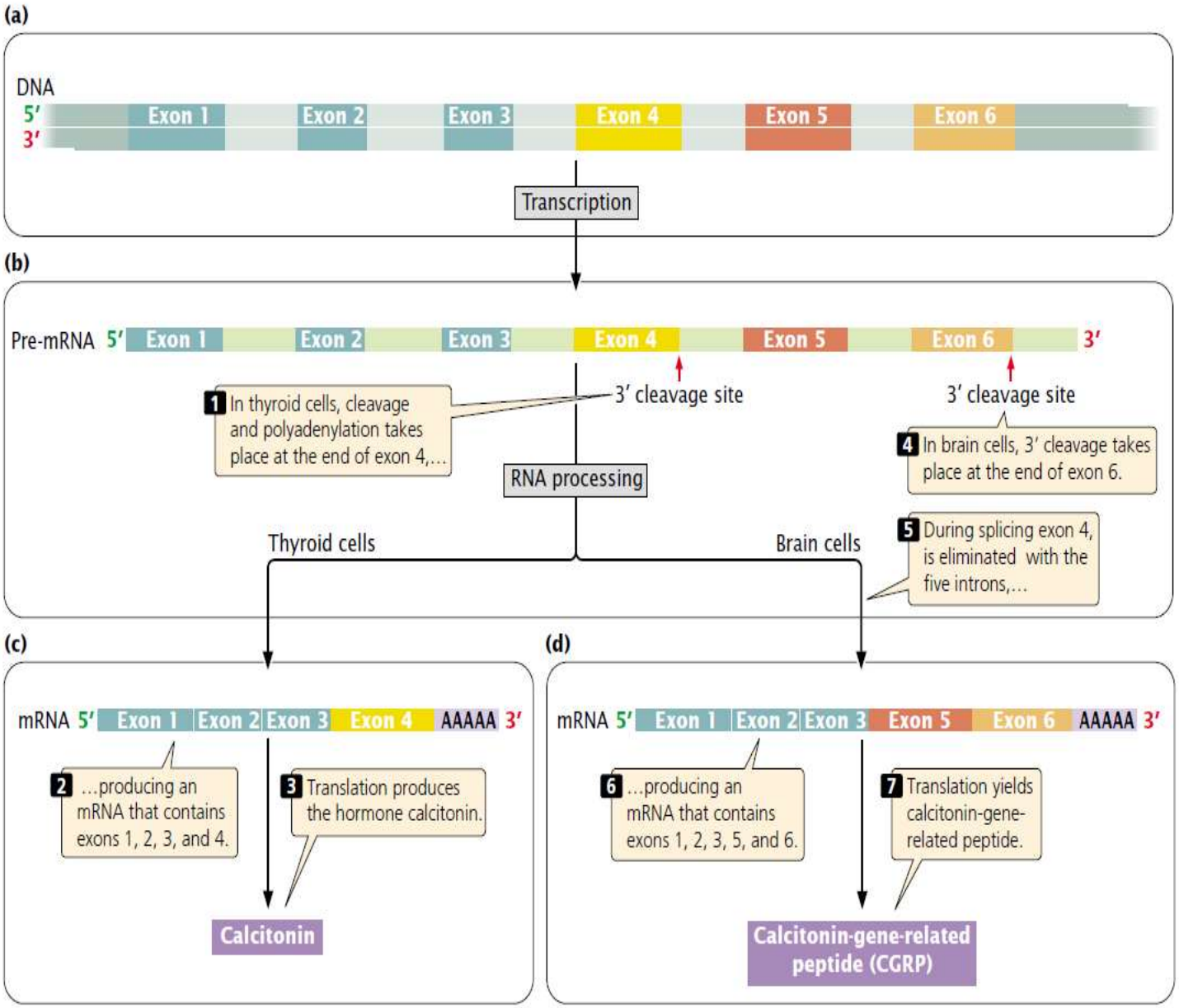


Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 393

Rashmi M C



14.17 Pre-mRNA encoded by the calcitonin gene undergoes alternative processing.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 394

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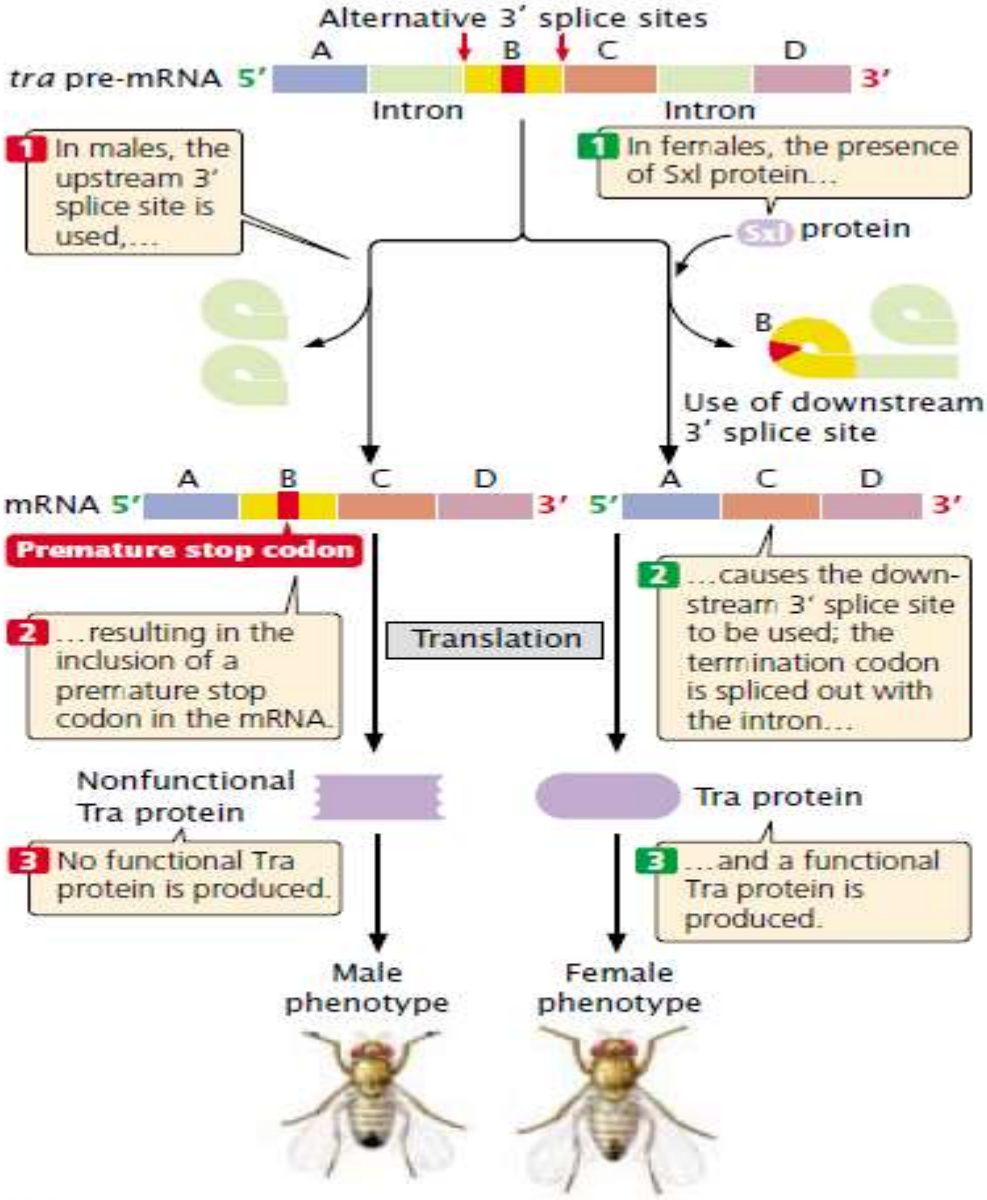

16.27 Alternative splicing of *tra* pre-mRNA. Two alternative 3′ splice sites are present.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 464

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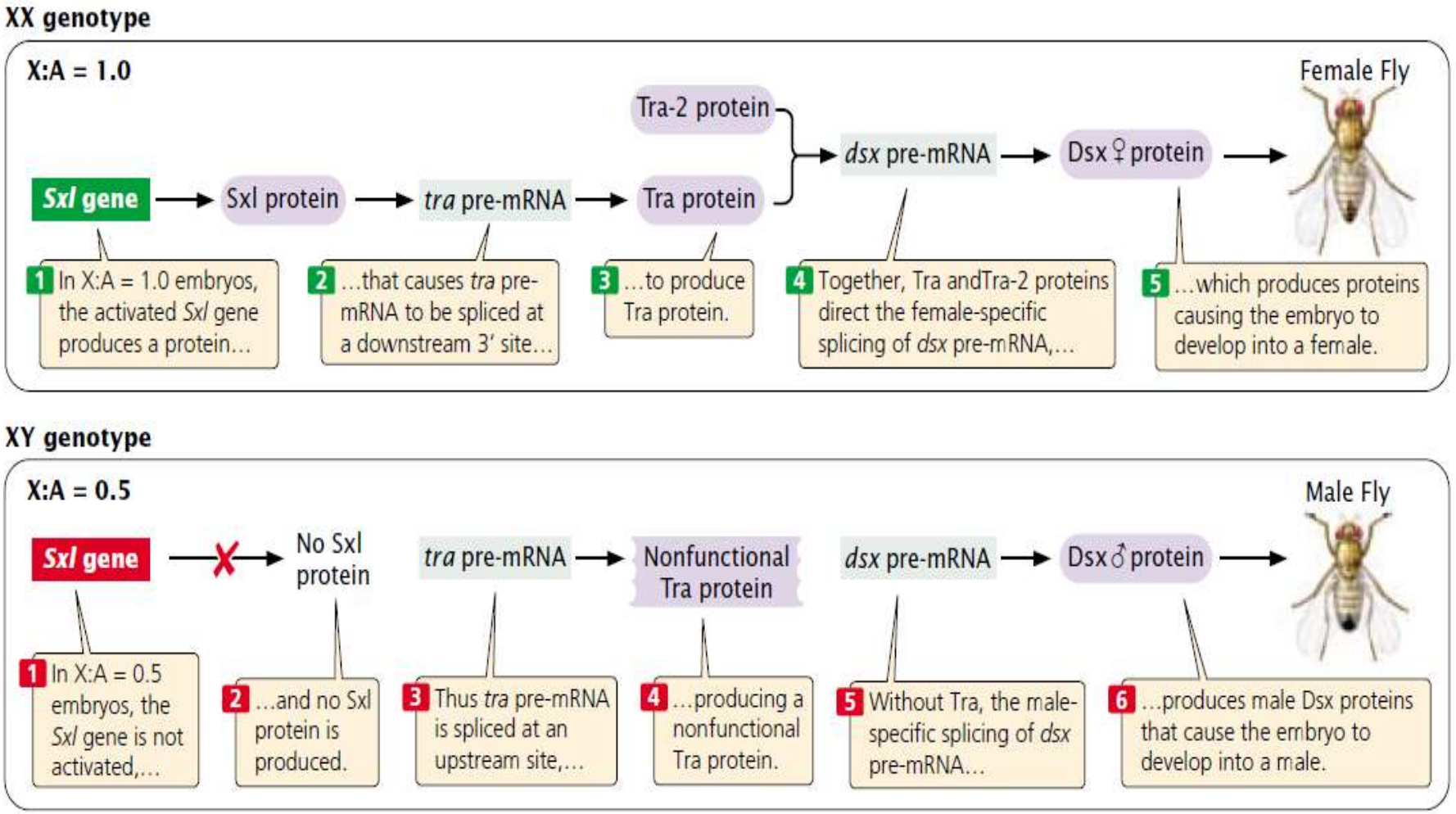
XX genotype
X:A = 1.0
Female Fly
Tra-2 protein
Sxl gene
Sxl protein
tra pre-mRNA
Tra protein
dsx pre-mRNA
Dsx♀ protein
1 In X:A = 1.0 embryos, the activated Sxl gene produces a protein...
2 ...that causes tra pre-mRNA to be spliced at a downstream 3' site...
3 ...to produce Tra protein.
4 Together, Tra andTra-2 proteins direct the female-specific splicing of dsx pre-mRNA,...
5 ...which produces proteins causing the embryo to develop into a female.
XY genotype
X:A = 0.5
Male Fly
Sxl gene
No Sxl protein
tra pre-mRNA
Nonfunctional Tra protein
dsx pre-mRNA
Dsx♂ protein
1 In X:A = 0.5 embryos, the Sxl gene is not activated,...
2 ...and no Sxl protein is produced.
3 Thus tra pre-mRNA is spliced at an upstream site,...
4 ...producing a nonfunctional Tra protein.
5 Without Tra, the male-specific splicing of dsx pre-mRNA...
6 ...produces male Dsx proteins that cause the embryo to develop into a male.

16.26 Alternative splicing controls sex determination in *Drosophila*.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 463

4. RNA editing:

- Changing the nucleotide sequence of RNA so that a mature RNA differs from that encoded by the genomic sequence
- 2 ways of RNA editing:
  - **Site specific base modification editing (Substitution editing)**
  - **Insertion-Deletion editing**

- **Site specific base modification editing (Substitution editing)**

Ex.1- Deamination

2 common deamination based RNA editing:

C →U editing: Found in human mRNA for apolipoprotein B

A → I editing

Ex.2- Transamination (U →C editing in Wilms Tumor gene)

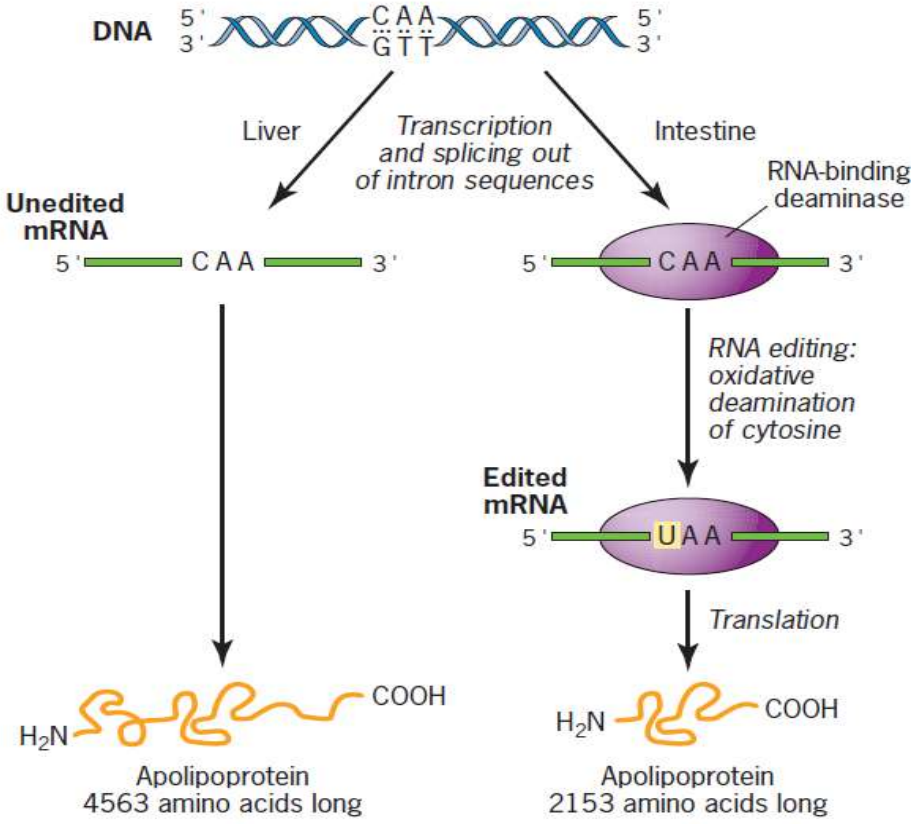

FIGURE 11.18 Editing of the apolipoprotein-B mRNA in the intestines of mammals.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 273

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Insertion- Deletion editing

- Nucleotides (Uridine containing nucleotides) are inserted into or deleted from specific region of an mRNA after transcription
- Editing reactions involve, cleavage, insertions or deletions and ligation
- These reactions are catalyzed by the **20S editosome** (a protein complex containing all of the enzymatic machinery necessary to catalyze editing)
- The sites in the pre-mRNA to be edited are defined by small RNAs that are complementary to edited RNA sequences
- These are commonly referred to as **guide RNAs (gRNAs)**

The gRNA has 3 domains:

1. The 5' region: which is complementary to the substrate pre-mRNA
2. The central domain: which contain the information necessary to insert and/or delete nucleotides in the pre-mRNA to make the edited sequence
3. The 3' end of the guide RNA: which is characterized by a poly U- tail

Depending on the information present in the template region of the gRNA uridylates may either be added to the pre-mRNA by a **terminal uridyl transferase** alternately uridylates may be deleted by an **uridyl exonuclease**

Following U insertion or deletion the 5' and 3' fragments of the pre-mRNA are ligated through the action of **editosome RNA ligase**

Consequences of RNA editing:

- Causes changes in function of edited transcripts as compared to the unedited transcripts
- Alters codons in mRNAs
- May alter splicing pattern by changing splice site
- May affect RNA degradation by modifying RNA sequences involved in nuclease recognition
- May affect RNA structure dependent activities that involve binding of RNA by proteins

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Stages of Editing:

gRNA / mRNA duplex formation

↓

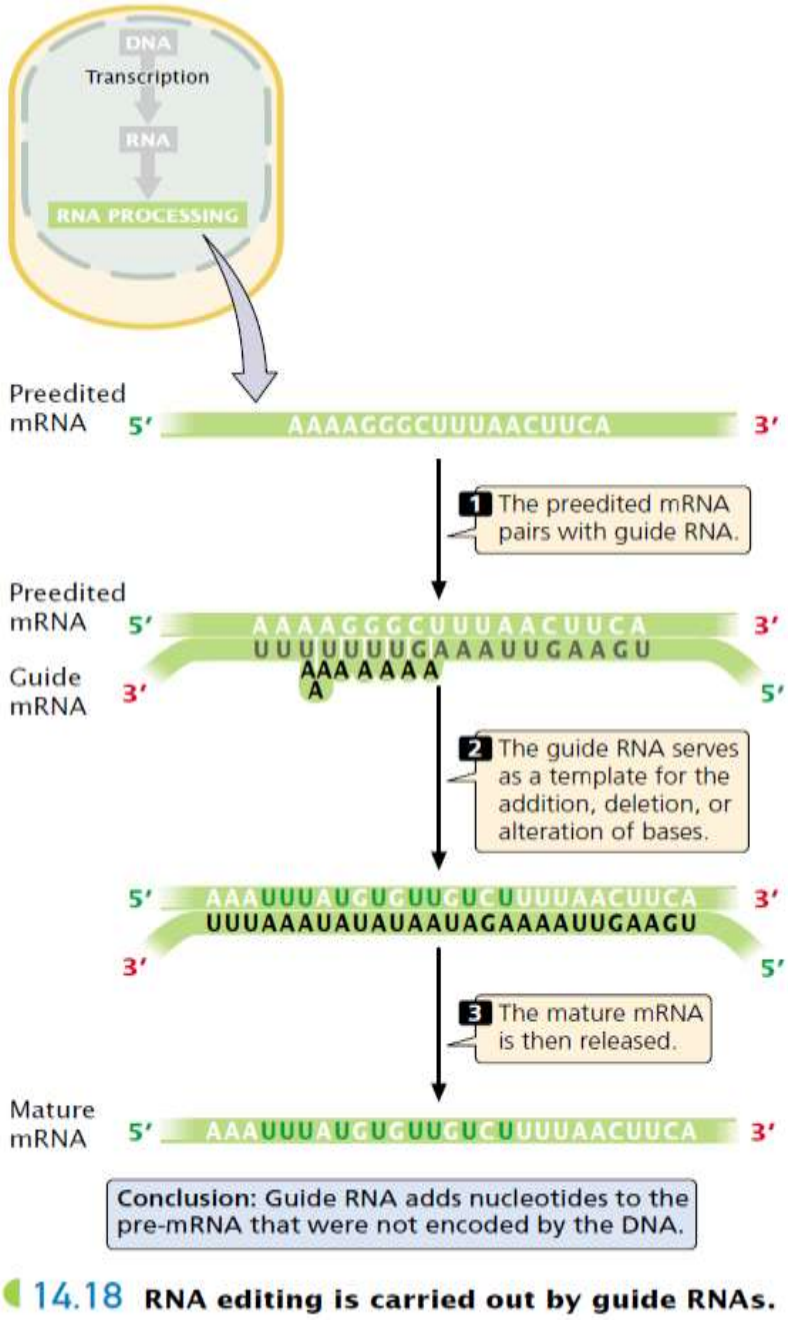
Cleavage of the mRNA at target site

↓

Addition/ deletion of U residue in mRNA

↓

Re-ligation of the two fragments of the newly edited mRNA


DNA
Transcription
RNA
RNA PROCESSING
Preedited mRNA
5′ AAAAGGGCUUUAACUUCA 3′
1 The preedited mRNA pairs with guide RNA.
Preedited mRNA
5′ AAAAGGGCUUUAACUUCA 3′
Guide mRNA
3′ UUUUUUUUGAAAUUGAAGU AAA AAAA A 5′
2 The guide RNA serves as a template for the addition, deletion, or alteration of bases.
5′ AAAUUUAUGUGUUGUCUUUUAACUUCA 3′
3′ UUUAAAUAUAUAAUAGAAAAUUGAAGU 5′
3 The mature mRNA is then released.
Mature mRNA
5′ AAAUUUAUGUGUUGUCUUUUAACUUCA 3′
Conclusion: Guide RNA adds nucleotides to the pre-mRNA that were not encoded by the DNA.

14.18 **RNA editing is carried out by guide RNAs.**

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no.395

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Processing of pre-rRNA:

In eukaryotes,

1. **Cutting and end trimming process**

- Involves the use of **endonucleases** (to cut at specific sites within a longer pre-rRNA) and **exonuclease** (to trim back from the new ends to make the mature product)

2. **Chemical modification of rRNA**

- Requires a class of small RNAs called **snoRNAs (Small nucleolar RNAs)**
- These snoRNAs primarily guide chemical modifications of other RNAs, mainly rRNAs, tRNAs and small nuclear RNAs
- 2 main classes of snoRNAs:
  - **C/D box snoRNAs**: are associated with methylation
  - **H/ACA box snoRNAs**: associated with Pseudouridylation
- These snoRNAs base pairs with the rRNA to create the double stranded region that is recognized as a substrate for methylation and Pseudouridylation

In prokaryotes,

Processing of pre-rRNA involves,

1. **Cleavage**
2. **Trimming**
3. **Methylation of 30S pre-rRNA**

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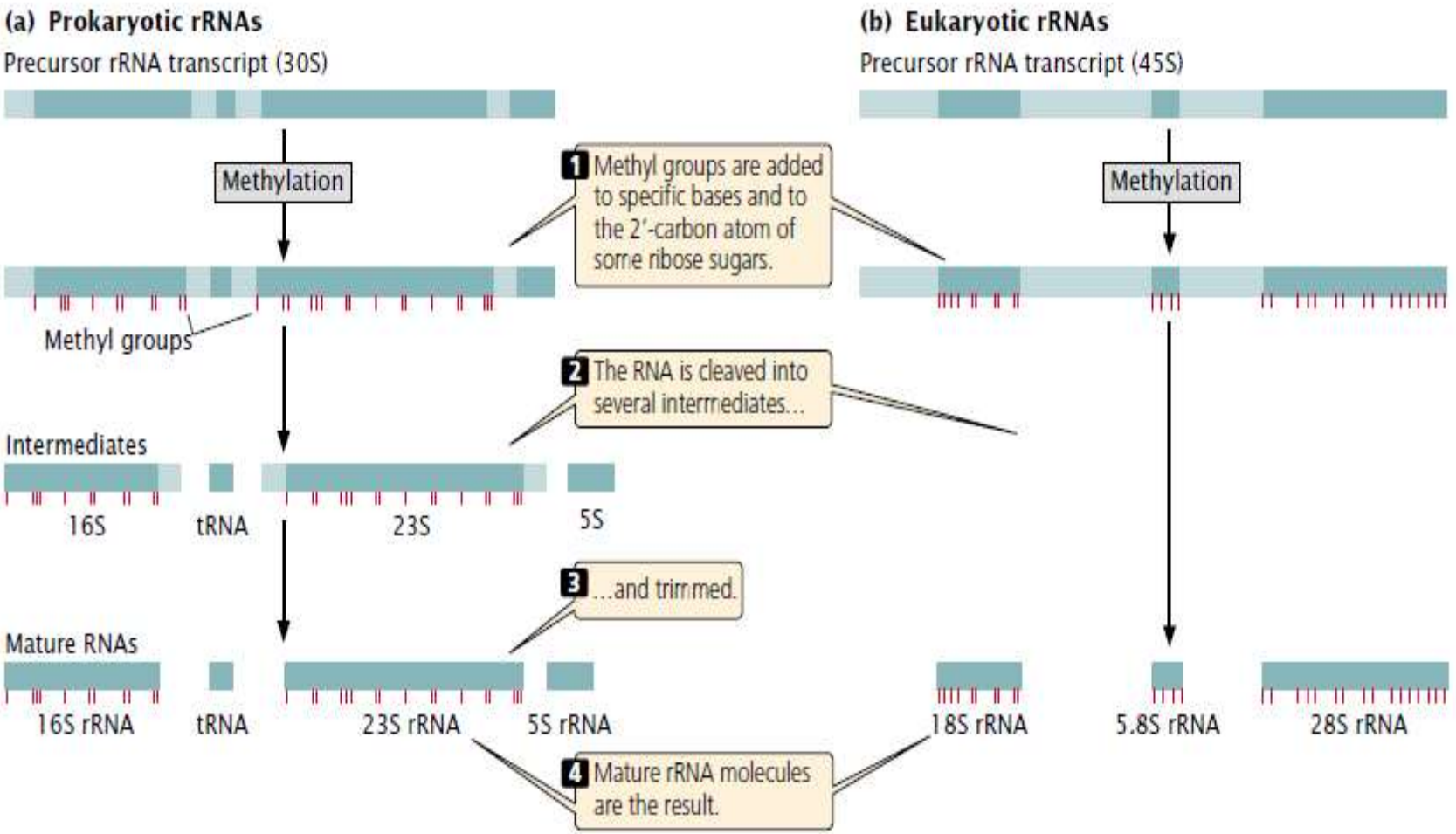


14.24 Ribosomal RNA is processed after transcription. Note that eukaryotic rRNA does not undergo trimming and that 5S rRNA is transcribed separately from the small eukaryotic rRNA gene.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no.400

Rashmi M G

Group I and Group II introns:

- These are self splicing introns
- Both are found in few prokaryotic genes also Present in few eukaryotic nuclear and organelles genes
- They perform splicing without the help of proteinaceous enzymes and acts as **ribozymes**
- These are self splicing RNA molecules that contain their own active site for intron removal and exon ligation
- These introns are distinguished by their mechanisms of splicing and unique structures
- It was first discovered by T. Cech during studies of splicing mechanism of the group I intron of pre-rRNA in ciliated protozoan ***Tetrahymena thermophila***

Group I introns

- Are found in the primary transcripts of some nuclear pre-rRNA forming genes and some organelle's genes
- These introns can move
- It migrates by DNA-mediated mechanism which is termed as **homing**

Group II introns

- Are found in primary transcripts of some organelle's genes
- These introns can move
- It migrates by RNA-mediated mechanism
- The protein coded by ORF also has reverse transcriptase activity apart from endonuclease activity
- The reverse transcriptase generates a DNA copy of the intron using 3'-OH of the double stranded break as a primer for synthesis and the mRNA of the intron as a template
- This process is called **retrohoming**

- Both Group I and Group II introns are **metalloenzymes**
- Requires divalent metal cations for activity (usually Mg^{2+} or Mn^{2+})
- Many Group I and Group II introns in organelle genes contain **open reading frame (ORF)** which codes for a protein called **maturase** that appears to play a role in splicing and also has endonuclease activity which allows the introns to be mobile

Rashmi M G

Both classes of self-splicing introns perform 2 consecutive transesterification reactions in the process of exon ligation

Neither class requires ATP for splicing

↓

Nucleophilic attack at the 5'-splice site by the 3'-OH of an exogenous (free) **guanosine** or **guanine nucleotide** (**GMP, GDP or GTP**)

This reaction adds the G nucleotide or nucleoside onto the 5' end of the intron and releases the 5' exon

↓

3'-OH of the 5' exon that is generated in first transesterification reaction then acts as a nucleophile at the 3' splice site of the intron

This results in precise excision of the intron

↓

Ligation of the exons without lariat formation

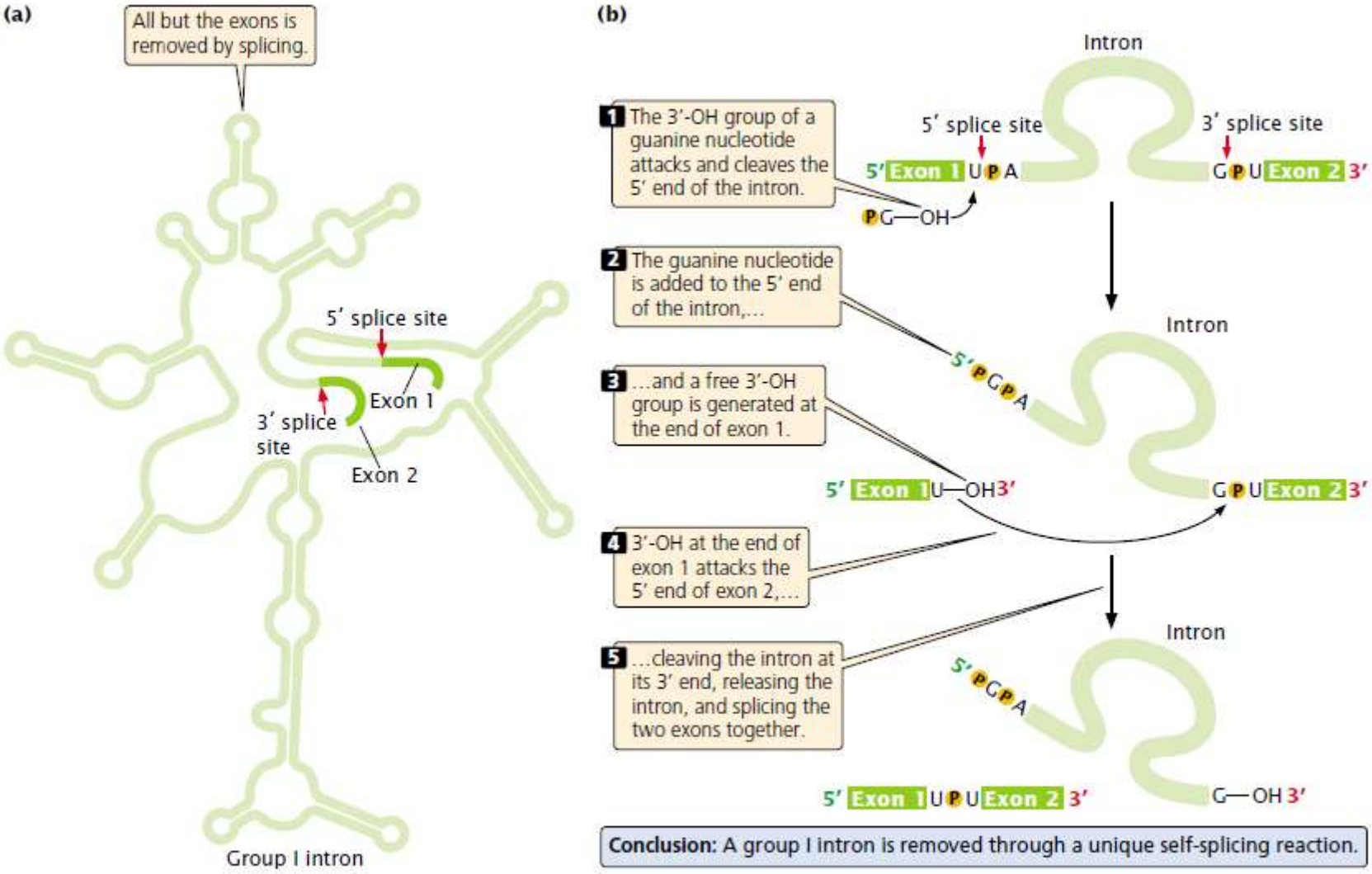
↓

The 2'-OH of a highly conserved A nucleotide (An internal nucleophile) of the intron attacks the 5' splice site

↓

Release of the 5' exon and formation of lariat structure, whose 5' end of the intron is covalently attached to the 2'-OH of the bulged A

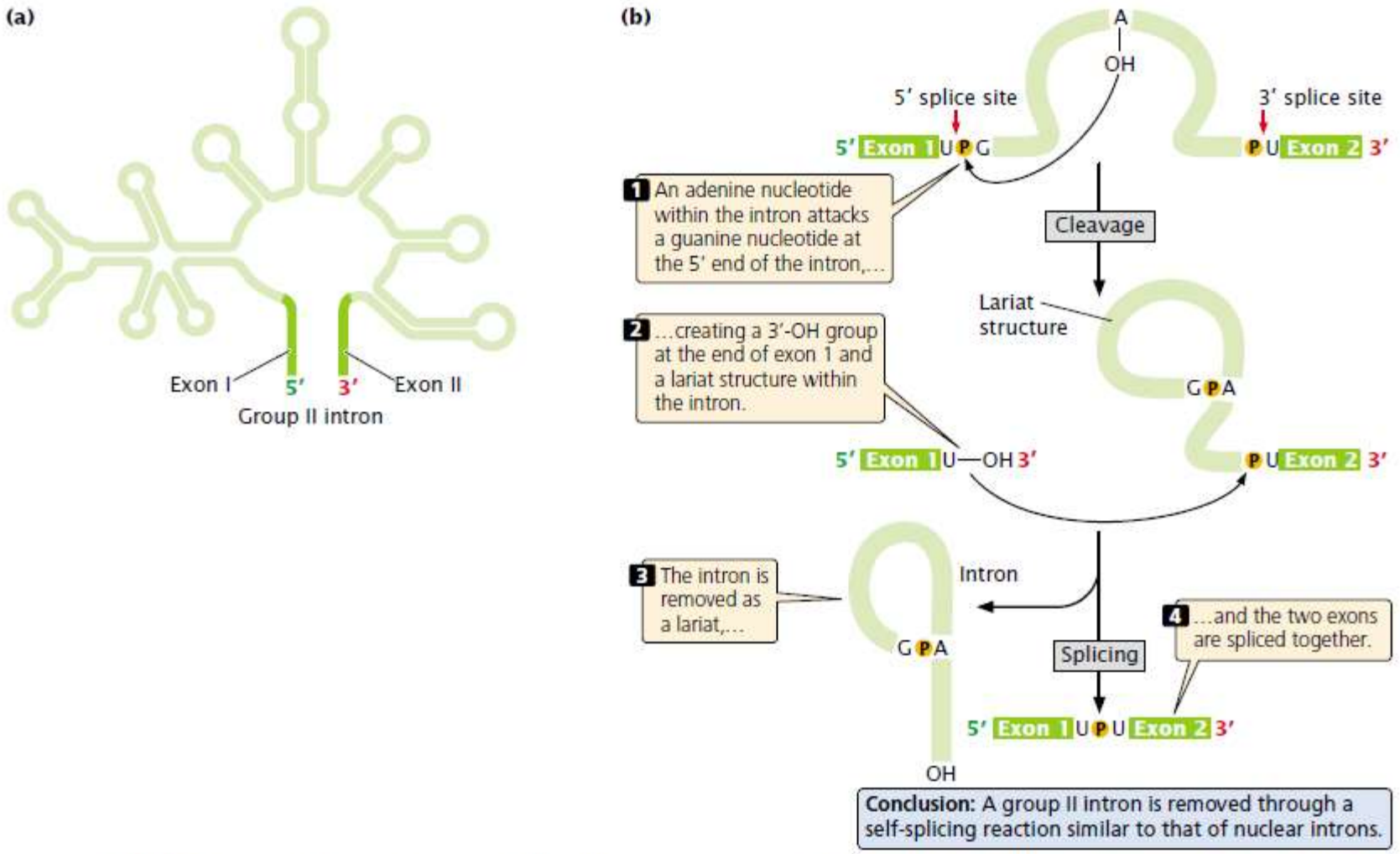
Rashmi M G


(a)
All but the exons is removed by splicing.
5′ splice site
Exon 1
3′ splice site
Exon 2
Group I intron
(b)
Intron
1 The 3′-OH group of a guanine nucleotide attacks and cleaves the 5′ end of the intron.
5′ splice site
3′ splice site
5′ Exon 1 U P A
G P U Exon 2 3′
P G—OH
2 The guanine nucleotide is added to the 5′ end of the intron,...
Intron
5′ P G P A
3 ...and a free 3′-OH group is generated at the end of exon 1.
5′ Exon 1 U—OH 3′
G P U Exon 2 3′
4 3′-OH at the end of exon 1 attacks the 5′ end of exon 2,...
Intron
5 ...cleaving the intron at its 3′ end, releasing the intron, and splicing the two exons together.
5′ P G P A
5′ Exon 1 U P U Exon 2 3′
G—OH 3′
Conclusion: A group I intron is removed through a unique self-splicing reaction.

14.14 Group I introns undergo self-splicing. (a) Secondary structure of a group I intron. (b) Self-splicing of a group I intron.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 391

Rashmi M G


(a)
Exon I
5′
3′
Exon II
Group II intron
(b)
A
OH
5′ splice site
3′ splice site
5′ Exon 1 U P G
P U Exon 2 3′
1 An adenine nucleotide within the intron attacks a guanine nucleotide at the 5′ end of the intron,…
Cleavage
Lariat structure
2 …creating a 3′-OH group at the end of exon 1 and a lariat structure within the intron.
G P A
5′ Exon 1 U—OH 3′
P U Exon 2 3′
3 The intron is removed as a lariat,…
Intron
G P A
4 …and the two exons are spliced together.
Splicing
5′ Exon 1 U P U Exon 2 3′
OH
Conclusion: A group II intron is removed through a self-splicing reaction similar to that of nuclear introns.

14.15 Group II introns undergo self-splicing by a different mechanism from that for group I introns. (a) Secondary structure of a group II intron. (b) Self-splicing of group II introns, which is similar to the splicing of nuclear introns.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 392

Rashmi M G

Processing of pre-tRNA:
Involves **cleavage > trimming > chemical modification and splicing**

tRNAs are commonly synthesized as precursor containing extra sequences that are removed by combinations of endonucleolytic and exonucleolytic activities

↓

5’ end of tRNA is generated by a cleavage action catalyzed by the enzyme **Ribonuclease P** (**RNase P**), a ribonucleoprotein wherein the RNA component serves as a ribozyme

↓

Generation of 3’ end in ***E coli*** involves endonuclease **RNase E/F** and exonuclease **RNase D**

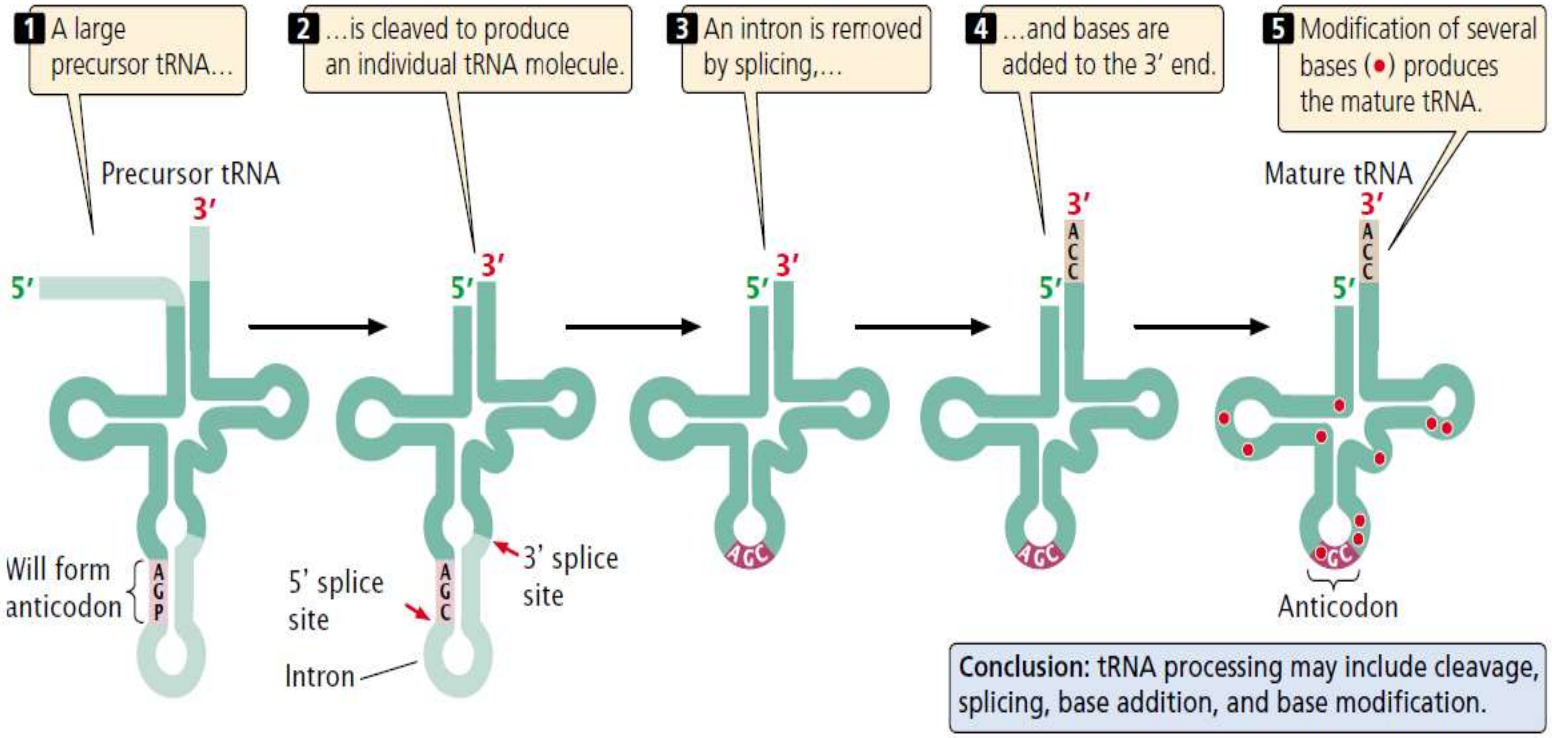
↓

RNase E makes cut at 3’ end then **RNase D** trims seven nucleotides from this new 3’ end

↓

One feature that is common in all tRNAs is the **triplet sequence CCA**, at the 3’ terminus
The triplet sequence is not coded in the genome, but added as a part of tRNA processing by **tRNA nucleotidyltransferase**

Rashmi M G


1 A large precursor tRNA...
2 ...is cleaved to produce an individual tRNA molecule.
3 An intron is removed by splicing,...
4 ...and bases are added to the 3' end.
5 Modification of several bases (•) produces the mature tRNA.
Precursor tRNA
Mature tRNA
5'
3'
Will form anticodon
A G P
5' splice site
3' splice site
A G C
Intron
A C C
Anticodon
Conclusion: tRNA processing may include cleavage, splicing, base addition, and base modification.

14.23 Transfer RNAs are processed in both bacterial and eukaryotic cells. Different tRNAs are modified in different ways. One example is shown here.

Source: Benjamin A Pierce, Genetics, A conceptual approach, Page no. 399

Rashmi M G

mRNA degradation

Prokaryotic mRNA:

- The average half life of bacterial mRNAs is only 1.5 minutes
- Degradation of mRNA occurs in the 3’ to 5’ direction
- Mediated by several endo- and exonuclease
- Endonuclease (**RNase E** and **RNase III**) make an internal cut in RNA molecules
- Exonuclease (**RNase II** and **polynucleotide phosphorylase, PNPase**) removes nucleotides sequentially from the 3’ end of an mRNA
- In ***E coli***, **RNase E** and **PNPase** along with **RNA helicase** are located within a multiprotein complex called **degradosome**

Eukaryotic mRNA

- The average half life of eukaryotic mRNA is 10-20 minutes in lower eukaryotes; to several hours in mammals
- Several degradation pathways of mRNA occur in both 5’ to 3’ direction and 3’ to 5’ direction
- Cytosolic mRNA are Degraded by 3 different pathways:
- **Deadenylation dependent pathway**: removal of poly (A) tail catalyzed by **deadenylase enzyme**
- Then deadenylated mRNA may either be decapped and degraded by 5’-3’ exonuclease or been degraded by a 3’-5’ exonuclease
- **Deadenylation independent pathway**: mRNAs are decapped and degraded by the 5’-3’ exonuclease
- **Endonucleolytic pathway**: does not involved decapping or Deadenylation

Rashmi M G

5’-3’ degradation pathway (Major)

- Deadenylation at the 3’ end triggers decapping at the 5’ end
- Decapping reaction occurs by cleavage of 1-2 bases from the 5’ end
- Removal of cap triggers the 5’-3’ degradation pathway in which mRNA is degraded rapidly from the 5’ end by the 5’-3’ exonuclease

3’-5’ degradation pathway

- Deadenylation of mRNA degraded by the 3’-5’ exonuclease activity
- **Exosome**, which is related to degradosome, degrades the mRNA in the 3’ to 5’ direction
- The Exosome is also found in the nucleus, where it degrades unspliced precursors to mRNA

Rashmi M G

mRNA surveillance: is a conserved mRNA degradation mechanism utilized by organisms to ensure fidelity and quality of mRNA molecules
2 most important mechanisms:

Nonsense mediated decay (NMD)

- Detection and decay of mRNA transcripts which contain premature termination codons
- It checks that mRNA have been properly synthesized and functions

Nonstop mediated mRNA decay

- Detection and decay of mRNA transcripts which lack in-frame stop codon
- These transcripts are identified during translation when the ribosome arrives at the 3’-end of the mRNA and stalls
- Exosome degrades the transcript